

AMENDMENT

Please amend the application without prejudice, without admission, without surrender of subject matter and without intention of creating any estoppel as to equivalents, as follows.

In the Claims

1. (Original) A diagnostic kit comprising at least one isolated and purified polypeptide comprising a WNV envelope (E) protein or an immunogenic fragment thereof having an native conformation or non-denatured structure whereby the E glycoprotein or the immunogenic fragment thereof is reactive with antibodies against WNV and cross-reactive with antibodies against a flavivirus.
2. (Original) The kit according to claim 1, wherein said E glycoprotein or fragment is from WNV isolate 2741.
3. (Original) The kit according to claim 1, wherein the amino acid sequence of the WNV E glycoprotein or immunogenic fragment thereof is the amino acid sequence encoded by SEQ ID NO.5.
4. (Original) The kit according to any one of claims 1-3, wherein said E glycoprotein or fragment thereof is part of a fusion protein.
5. (Original) The kit according to claim 4, wherein the fusion protein comprises a maltose binding protein or thioredoxin and said E glycoprotein.
6. (Original) The kit according to claim 1, wherein said flavivirus is DENV.
7. (Original) The kit according to claim 1, wherein said flavivirus is JEV.
8. (Original) The kit according to claim 1, wherein said flavivirus is SLEV.

9. (Original) A method for detecting a WNV infection in a subject suspected of having said infection comprising the steps of (a) contacting a biological sample from the subject with an isolated and substantially purified polypeptide comprising a WNV envelope (E) protein or an immunogenic fragment thereof having a native conformation or non-denatured structure whereby the E glycoprotein or the immunogenic fragment thereof is reactive with antibodies against WNV, and (b) detecting antibodies against WNV that have reacted with the WNV E protein, wherein detection of the antibodies indicates a WNV infection.

10. (Original) A method for detecting a flavivirus infection comprising the steps of (a) contacting a sample from a subject suspected of having said infection with an isolated and substantially purified polypeptide comprising a WNV envelope (E) protein or an immunogenic fragment thereof having a native conformation or non-denatured structure whereby the E glycoprotein or the immunogenic fragment thereof is cross-reactive with antibodies against a flavivirus, and (b) detecting antibodies that have reacted with the WNV E protein, wherein detection of antibodies indicates a flavivirus infection.

11. (Original) A method for detecting a protective immune response in a subject comprising the step of contacting a biological sample from said subject with an isolated and substantially purified polypeptide comprising a WNV envelope (E) protein or an immunogenic fragment thereof whereby the E glycoprotein or the immunogenic fragment thereof having a native conformation or non-denatured structure is reactive with protective antibodies against WNV and cross-reactive with protective antibodies against a flavivirus.

12. (Currently Amended) The methods ~~according to~~ as in one of claims 9-11, wherein said E glycoprotein or fragment thereof is comprised of SEQ ID NO.6.

13. (Currently Amended) The methods ~~according to~~ as in one of claims 9-11, wherein the amino acid sequence of the WNV E glycoprotein or fragment thereof is encoded by SEQ ID NO.5.

14. (Original) The method according to claims 9-11, wherein said E glycoprotein or fragment thereof is part of a fusion protein.

15. (Original) The method according to claim 14, wherein the fusion protein comprises a maltose binding protein or thioredoxin and said E glycoprotein or fragment thereof.

16. (Currently Amended) The method ~~s according to~~ as in one of claims 9-11, wherein said flavivirus is DENV.

17. (Currently Amended) The method ~~s according to~~ as in one of claims 9-11, wherein said flavivirus is JEV.

18. (Currently Amended) The method ~~s according to~~ as in one of claims 9-11, wherein said flavivirus is SLEV.

19. (Original) A method for detecting a first antibody to a flavivirus from a biological specimen of a subject suspected of being infected by said flavivirus comprising the steps of:

(a) contacting the biological specimen with a substantially pure WNV envelope (E) protein or an immunogenic fragment thereof having a native conformation and non-denatured structure under conditions to form a complex between the E glycoprotein and the first antibody, if present, that recognizes and binds the E glycoprotein,

(b) detecting the first antibody of said complex,
wherein the E glycoprotein is reactive to an antibody against a WNV and cross-reactive to an antibody against a flavivirus other than a WNV.

20. (Original) The method according to claim 19, wherein said E glycoprotein is coupled to a microsphere.

21. (Original) The method according to claim 19, wherein step (b) comprises the steps of:

(b_i) contacting said complex between said E glycoprotein and said first antibody with a second antibody reactive against said first antibody,

(b_{ii}) detecting the second antibody,
wherein detecting the second antibody infers detecting the first antibody.

22. (Original) The method according to claim 21, wherein the second antibody includes a fluorescent marker.

23. (Original) The method according to claim 21, wherein the step of detecting the second antibody further comprises the step of immunofluorescence detection.

24. (Original) The method according to claim 21, wherein the second antibody is coupled to an enzyme which can be assayed.

25. (Original) The method according to claim 24, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

26. (Original) The method according to claim 19, wherein said flavivirus is SLEV.

27. (Original) The method according to claim 19, wherein said flavivirus is WNV.

28. (Original) The method according to claim 19, wherein the biological specimen is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

29. (Original) The method according to claim 28, wherein the biological specimen is from a human.

30. (Original) A method according to claim 19, wherein the E glycoprotein is a fusion protein.

31. (Original) A method according to claim 30, wherein said fusion protein comprises a maltose binding protein or thioredoxin and WNV E glycoprotein or subfragment thereof.

32. (Original) A method for detecting a recent or ongoing flavivirus infection using a microsphere immunoassay to detect an IgM antibody against a flavivirus in a biological specimen comprising the steps of:

(a) contacting the biological specimen with anti-IgG antibodies to form IgG immune complexes,

(b) removing said complexes to form a biological specimen comprising IgM antibodies but lacking IgG antibodies,

(c) contacting the biological specimen with a microsphere coupled to a substantially pure WNV E glycoprotein having a native conformation or non-denatured structure to form a microsphere mixture under conditions sufficient to form a binding complex between the E glycoprotein and a IgM antibody whereby the E glycoprotein is reactive to antibodies against WNV and cross-reactive to antibodies against a flavivirus,

(d) contacting the microsphere mixture with a detection reagent capable of detecting a IgM antibody,

(e) detecting the detection reagent,
whereby detection of the detection reagent indicates a recent or ongoing flavivirus infection.

33. (Original) A method for detecting a protective immune response to a flavivirus using a microsphere immunoassay to detect an IgG antibody against a flavivirus in a biological specimen comprising the steps of:

(a) contacting the biological specimen with anti-IgM antibodies to form IgM immune complexes,

(b) removing said complexes to form a biological specimen comprising IgG antibodies but lacking IgM antibodies,

(c) contacting the biological specimen with a microsphere coupled to a substantially pure WNV E glycoprotein having a native conformation or non-denatured structure to form a

microsphere mixture under conditions sufficient to form a binding complex between the E glycoprotein and a IgG antibody whereby the E glycoprotein is reactive to antibodies against WNV and cross-reactive to antibodies against a flavivirus,

(d) contacting the microsphere mixture with a detection reagent capable of detecting a IgG antibody,

(e) detecting the detection reagent,

whereby detection of the detection reagent indicates a protective immune response to a flavivirus.

~~24-34.~~ (Currently Amended) The method according to claim 19, wherein said flavivirus is JEV.

35. (Currently Amended) The methods ~~according to~~ as in any one of claims 32 and 33, wherein said flavivirus is selected from the group consisting of WNV, JEV, SLEV, and DENV.

36. (Currently Amended) The method-s ~~according to~~ as in any one of claims 32 and 33, wherein said biological specimen is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

37. (Original) The method according to claim 32, wherein the detection reagent comprises an anti-IgM antibody coupled to a fluorescent tag.

38. (Original) The method according to claim 33, wherein the detection reagent comprises an anti-IgG antibody coupled to a fluorescent tag.

39. (Original) The method according to claim 32, wherein the detection reagent comprises an anti-IgM antibody coupled to an enzyme.

40. (Original) The method according to claim 33, wherein the detection reagent comprises an anti-IgG antibody coupled to an enzyme.

41. (Currently Amended) The methods ~~according to~~ as in any one of claims 39 and 40, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

42. (Currently Amended) The methods ~~according to~~ as in any one of claims 37 and 38, wherein step (e) comprises the step of immunofluorescence detection of said fluorescent tag of said antibody of said detection reagent.

43. (Currently Amended) The methods ~~s-according to~~ as in any one of claims 32 and 33, wherein the step of removing said complexes comprises the step of centrifugation.

44. (Original) A method for rapidly detecting an antibody against a flavivirus antigen comprising the steps of:

(a) contacting a biological sample with a microsphere suspension, each microsphere coupled to a substantially pure WNV E glycoprotein having a native conformation or non-denatured structure whereby each E glycoprotein is reactive to antibodies against WNV and cross-reactive to antibodies against a flavivirus,

(b) incubating the microsphere suspension under conditions sufficient to increase reaction kinetics to promote the binding of an anti-WNV or anti-flavivirus antibody to the E glycoproteins,

(c) contacting the microsphere suspension with a detection reagent capable of detecting an antibody against a WNV or a flavivirus,

(d) detecting the detection reagent,

wherein detection of the detection reagent indicates an antibody against a WNV or a flavivirus in the biological sample.

45. (Original) The method according to claim 44, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

46. (Original) The method according to claim 45, wherein the biological sample is 10-20 microliters.

47. (Original) The method according to claim 44, wherein the conditions sufficient to increase the reaction kinetics according to step (b) comprises an incubation temperature of 37°C, and incubation time of about 30 minutes, and motion.

48. (Original) The method according to claim 44, wherein the detection reagent comprises a polyvalent antibody coupled to a fluorescent tag, the polyvalent antibody comprising at least anti-IgG and anti-IgM antibodies.

49. (Original) The method according to claim 44, wherein the detection reagent comprises a polyvalent antibody coupled to an enzyme, the polyvalent antibody comprising at least anti-IgG and anti-IgM antibodies.

50. (Original) The method according to claim 49, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

51. (Original) The method according to claims 48, wherein step (d) comprises the step of immunofluorescence detection of said fluorescent tag of said antibody of said detection reagent.

52. (Original) The methods according to claim 44, wherein said flavivirus is selected from the group consisting of WNV, JEV, SLEV, and DENV.

53. (Original) A method for the detection of a flavivirus infection in a biological specimen comprising the steps of:

(a) obtaining a suspension of microspheres each coupled to a substantially pure WNV E glycoprotein having a native conformation or non-denatured structure wherein the WNV E glycoprotein is reactive with antibodies against WNV and cross-reactive with antibodies against a flavivirus;

(b) performing a microsphere immunoassay;

(c) obtaining a result indicating either the presence or absence of an antibody against the flavivirus,

wherein the presence of an antibody against the flavivirus indicates a flavivirus infection.

54. (Original) The method according to claim 53, wherein the microsphere immunoassay is a Luminex-based test.

55. (Original) The method according to claim 54, wherein the microsphere immunoassay is a lateral flow test.

56. (Original) The method according to claim 55, wherein the microsphere immunoassay is an agglutination test.

57. (Original) The method according to claim 53, wherein the microsphere immunoassay is a lateral flow immunoassay

58. (Original) The method according to claim 53, wherein the microsphere immunoassay is automated.

59. (Original) The method according to claim 53, wherein the flavivirus is selected from the group consisting of WNV, JEV, SLEV, and DENV.

60. (Original) A method for the transfer of information obtained as a result of carrying out the methods of any of claims 1, 9, 10, 11, 19, 32, 33, 44, or 53.

61. (Original) A diagnostic kit comprising isolated and purified WNV E glycoprotein or an immunogenic fragment thereof, wherein, the WNV E glycoprotein is reactive with antibodies against WNV, as well as with antibodies against at least one flavivirus that is other than WNV, and the WNV E glycoprotein has its native conformation or is non-denatured, and the immunogenic fragment contains at least an epitope of native WNV E in native conformation or that is non-denatured, whereby the immunogenic fragment is reactive with antibodies against WNV, as well as with antibodies against at least one other flavivirus.

62. (Original) A diagnostic kit comprising an isolated or purified WNV E glycoprotein or immunogenic fragment thereof, wherein, the WNV E glycoprotein is reactive with antibodies against WNV, as well as with antibodies against at least two other flaviviruses that are other than WNV, and the WNV E glycoprotein has its native conformation or is non-denatured, and the immunogenic fragment contains at least an epitope of native WNV E in native conformation or that is non-denatured, whereby the immunogenic fragment is reactive with antibodies against WNV, as well as with antibodies against at least two other flavivirus.

63. (Original) A diagnostic kit comprising isolated and purified WNV E glycoprotein or an immunogenic fragment thereof, wherein, the WNV E glycoprotein is reactive with antibodies against WNV, as well as with antibodies against at least three other flaviviruses that are other than WNV, and the WNV E glycoprotein has its native conformation or is non-denatured, and the immunogenic fragment contains at least an epitope of native WNV E in native conformation or that is non-denatured, whereby the immunogenic fragment is reactive with antibodies against WNV, as well as with antibodies against at least three other flavivirus.

64. (Original) The diagnostic kit according to claim 61, wherein the at least one other flavivirus is selected from the group consisting of JEV, SLEV, and DENV.

65. (Original) The diagnostic kit according to claim 62, wherein the at least two other flaviviruses are selected from the group consisting of JEV, SLEV, and DENV.

66. (Original) The diagnostic kit according to claim 63, wherein the at least three other flaviviruses are selected from the group consisting of JEV, SLEV, and DENV.

67. (Currently Amended) The kit ~~according to~~ as in one of claims 61-63, wherein the WNV E glycoprotein or fragment thereof is so purified that a diagnostic assay employing the WNV E glycoprotein or fragment thereof can detect flavivirus within about 3 hours.

68. (Currently Amended) The kit ~~according to~~ as in one of claims 61-63, wherein the WNV E glycoprotein or fragment thereof is so purified that a diagnostic assay employing the WNV E glycoprotein or fragment thereof can detect flavivirus within about 10 minutes.

69. (Original) A diagnostic kit comprising at least one isolated and purified polypeptide comprising a WNV NS5 protein or an immunogenic fragment thereof having an native conformation or non-denatured structure whereby the NS5 protein or the immunogenic fragment thereof is specifically reactive with antibodies against WNV but not detectably cross-reactive with antibodies against a flavivirus other than WNV.

70. (Original) The kit according to claim 69, wherein said NS5 protein or fragment thereof is from WNV isolate 2741.

71. (Original) The kit according to claim 69, wherein the amino acid sequence of the WNV NS5 protein or fragment thereof is the amino acid sequence encoded by the NS5.

72. (Original) The kit according to any one of claims 69-71, wherein said NS5 protein or fragment thereof is part of a fusion protein.

73. (Original) The kit according to claim 72, wherein the fusion protein comprises a maltose binding protein or thioredoxin and said NS5 protein.

74. (Original) A method for detecting a WNV infection in a subject suspected of having said infection comprising the steps of (a) contacting a biological sample from the subject

with an isolated and substantially purified polypeptide comprising a WNV NS5 protein or an immunogenic fragment thereof having a native conformation or non-denatured structure whereby the NS5 protein or the immunogenic fragment thereof is specifically reactive with anti-WNV antibodies but not detectably cross-reactive with antibodies against a flavivirus other than WNV, and (b) detecting anti-WNV antibodies that have reacted with the WNV NS5 protein, wherein detection of the anti-WNV antibodies indicates a WNV infection.

75. (Original) A method for detecting a protective immune response in a subject comprising the step of contacting a biological sample from said subject with an isolated and substantially purified polypeptide comprising a WNV NS5 protein or an immunogenic fragment thereof whereby the E glycoprotein or the immunogenic fragment thereof having a native conformation or non-denatured structure is specifically reactive with protective antibodies against WNV with no detectable cross-reactivity with protective antibodies against a flavivirus other than WNV.

76. (Currently Amended) The methods ~~according to~~ as in one of claims 74-75, wherein said NS5 protein or fragment thereof is from WNV isolate 2741.

77. (Currently Amended) The method ~~s according to~~ as in one of claims 74-75, wherein the amino acid sequence of the WNV NS5 protein or fragment thereof is the amino acid sequence encoded by the NS5 protein encoding DNA sequence of Genbank accession No. AF 404756, or a fragment thereof.

78. (Original) The method according to any one of claims 74-75, wherein said NS5 protein or fragment thereof is part of a fusion protein.

79. (Original) The method according to claim 78, wherein the fusion protein comprises a maltose binding protein or thioredoxin and said NS5 protein or fragment thereof.

80. (Original) A method for detecting a first antibody to a WNV from a biological specimen of a subject suspected of being infected by said WNV comprising the steps of:

(a) contacting the biological specimen with a substantially pure WNV NS5 protein or an immunogenic fragment thereof having a native conformation and non-denatured structure under conditions to form a complex between the NS5 protein and the first antibody, if present, that recognizes and binds the NS5 protein,

(b) detecting the first antibody of said complex,
wherein the NS5 protein is not detectably cross-reactive to an antibody against a flavivirus other than a WNV.

81. (Original) The method according to claim 80, wherein said NS5 protein is coupled to a microsphere, adsorbed to nitrocellulose paper, or dried to nitrocellulose paper.

82. (Original) The method according to claim 80, wherein step (b) comprises the steps of:

(b_i) contacting said complex between said NS5 protein and said first antibody with a second antibody reactive against said first antibody,

(b_{ii}) detecting the second antibody,
wherein detecting the second antibody infers detecting the first antibody.

83. (Original) The method according to claim 82, wherein the second antibody includes a fluorescent marker or the second antibody is bound to colloidal gold or polystyrene microspheres..

84. (Original) The method according to claim 82, wherein the step of detecting the second antibody further comprises the step of immunofluorescence detection.

85. (Original) The method according to claim 82, wherein the second antibody is coupled to an enzyme which can be assayed.

86. (Original) The method according to claim 85, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

87. (Original) The method according to claim 80, wherein the biological specimen is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

88. (Original) The method according to claim 87, wherein the biological specimen is from a human.

89. (Original) A method according to claim 80, wherein the NS5 protein is a fusion protein.

90. (Original) A method according to claim 89, wherein said fusion protein comprises a maltose binding protein or thioredoxin and WNV NS5 or subfragment thereof.

91. (Original) A method for rapidly detecting an anti-WNV antibody comprising the steps of:

(a) contacting a biological sample with a microsphere suspension, each microsphere coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against WNV but not detectably cross-reactive with antibodies against a flavivirus other than WNV,

(b) incubating the microsphere suspension under conditions sufficient to increase reaction kinetics to promote the binding of an anti-WNV antibody to the NS5 proteins,

(c) contacting the microsphere suspension with a detection reagent capable of detecting an anti-WNV antibody,

(d) detecting the detection reagent,
wherein detection of the detection reagent indicates the presence an anti-WNV in the biological sample.

92. (Original) The method according to claim 91, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

93. (Original) The method according to claim 92, wherein the biological sample is 10-20 microliters.

94. (Original) The method according to claim 91, wherein the conditions sufficient to increase the reaction kinetics according to step (b) comprises an incubation temperature of 37°C, and incubation time of about 30 minutes, and motion.

95. (Original) The method according to claim 91, wherein the detection reagent comprises a polyvalent antibody coupled to a fluorescent tag.

96. (Original) The method according to claim 91, wherein the detection reagent comprises a polyvalent antibody coupled to an enzyme.

97. (Original) The method according to claim 96, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

98. (Original) The method according to claims 95, wherein step (d) comprises the step of immunofluorescence detection of said fluorescent tag of said polyvalent antibody of said detection reagent.

99. (Original) A method for the detection of a WNV infection in a biological specimen comprising the steps of:

- (a) obtaining a suspension of microspheres each coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure wherein the WNV NS5 protein is specifically reactive with anti-WNV antibodies but not detectably cross-reactive with antibodies against a flavivirus;
- (b) performing a microsphere immunoassay;
- (c) obtaining a result indicating either the presence or absence of an anti-WNV antibody,

wherein the presence of an anti-WNV antibody indicates a WNV infection.

100. (Original) The method according to claim 99, wherein the microsphere immunoassay is a Luminex-based test.

101. (Original) The method according to claim 99, wherein the microsphere immunoassay is a lateral flow test.

102. (Original) The method according to claim 99, wherein the microsphere immunoassay is an agglutination test.

103. (Original) The method according to claim 99, wherein the microsphere immunoassay is a strip test.

104. (Original) The method according to claim 99, wherein the microsphere immunoassay is automated.

105. (Original) A method for the transfer of information obtained as a result of carrying out the methods of any of claims 74, 75, 80, 91, or 99.

106. (Currently Amended) A method for discriminating between whether (1) a host has an ongoing WNV infection or (2) a host has been vaccinated with a killed-flavivirus vaccine wherein the host in the case of (1) has both anti-E glycoprotein antibodies and anti-NS5 antibodies but in the case of (2) has anti-E glycoprotein but not anti-NS5 antibodies comprising the steps of:

(a) carrying out a first reaction comprising the steps of (i) contacting a biological sample from the host with a first detection reagent for the detection of anti-E glycoprotein antibodies, (ii) detecting said first detection reagent to provide either a positive or negative signal wherein a positive signal indicates the presence of anti-E glycoprotein antibodies and a negative signal indicates the absence of anti-E glycoprotein antibodies;

(b) carrying out a second reaction comprising the steps of (i) contacting a biological sample from the host with a second detection reagent for the detection of anti-NS5 antibodies, (ii) detecting said second detection reagent to provide either a positive or negative signal wherein a positive signal indicates the presence of anti-NS5 antibodies and a negative signal indicates the absence of anti-NS5 antibodies;

(c) comparing the results of the first and second reactions wherein the following ~~may~~ may be true: (i) a positive signal for anti-E glycoprotein antibody and a positive signal for anti-NS5 antibody indicates that the host has an ongoing WNV infection and (ii) a positive signal for anti-E glycoprotein antibody and a negative signal for anti-NS5 antibody indicates that the host does not have an ongoing WNV infection but may have been vaccinated with a killed-flavivirus vaccine.

107. (Original) A method for detecting a recent or ongoing WNV infection in a host comprising the steps of:

(a) carrying out a first reaction comprising the steps of (i) contacting a biological sample from the host with a first detection reagent for the detection of anti-E glycoprotein antibodies, (ii) detecting said first detection reagent to provide either a positive or negative signal wherein a positive signal indicates the presence of anti-E glycoprotein antibodies and a negative signal indicates the absence of anti-E glycoprotein antibodies;

(b) carrying out a second reaction comprising the steps of (i) contacting a biological sample from the host with a second detection reagent for the detection of anti-NS5 antibodies, (ii) detecting said second detection reagent to provide either a positive or negative signal wherein a positive signal indicates the presence of anti-NS5 antibodies and a negative signal indicates the absence of anti-NS5 antibodies;

(c) comparing the results of the first and second reactions wherein the following may be true: (i) a positive signal for anti-E glycoprotein antibody and a positive signal for anti-NS5 antibody indicates that the host has a recent or ongoing WNV infection and (ii) a positive signal for anti-E glycoprotein antibody but a negative signal for anti-NS5 antibody indicates that the host does not have a recent or ongoing WNV infection.

108. (Currently Amended) The method ~~according to~~ as in one of claims 106-107 wherein the first detection reagent comprises a WNV E glycoprotein or fragment thereof from WNV isolate 2741 and wherein the amino acid sequence of SEQ ID NO.6.

109. (Original) The method according to claim 108, wherein the step of (a)(i) detecting said first detection reagent further comprises contacting with at least one antibody against the WNV E glycoprotein.

110. (Original) The method according to claim 109, wherein the at least one antibody against the WNV E glycoprotein further comprises a detectable signal.

111. (Original) The method according to claim 110, wherein the detectable signal is selected from the group consisting of a fluorescent label, an enzyme, and radioactive marker, or color from a colloidal gold or a colored polystyrene microsphere.

112. (Original) The method according to claim 111, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

113. (Original) The method according to claims 106-107, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

114. (Original) The method according to claims 106-107, wherein the host is a human.

115. (Original) The method according to claims 106-107, wherein the host is a horse.

116. (Original) The method according to claim 109, wherein the fusion protein comprises a maltose binding protein or thioredoxin and said WNV E glycoprotein or fragment thereof.

117. (Original) The method according to claims 106-107 wherein the second detection reagent comprises a WNV NS5 protein or fragment thereof from WNV isolate 2741 and wherein the amino acid sequence of the WNV NS5 protein or fragment thereof is the amino acid sequence of SEQ ID NO.6.

118. (Original) The method according to claim 106-107, wherein the step of (a)(i) detecting said second detection reagent further comprises contacting with at least one antibody against the WNV NS5 protein.

119. (Original) The method according to claim 118, wherein the at least one antibody against the WNV NS5 protein further comprises a detectable signal.

120. (Original) The method according to claim 119, wherein the detectable signal is selected from the group consisting of a fluorescent label, an enzyme, and a radioactive marker.

121. (Original) The method according to claim 111, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

122. (Original) The method according to claims 106-107 wherein the first detection reagent comprises a WNV E glycoprotein or fragment thereof from WNV isolate 2741 and wherein the amino acid sequence of the WNV E glycoprotein or fragment thereof is the amino acid sequence encoded by the E glycoprotein encoding DNA sequence of Genbank accession No. AF 404756 or a fragment thereof.

123. (Original) The kit according to claim 1, wherein the amino acid sequence of the WNV E glycoprotein or fragment thereof is the amino acid sequence encoded by SEQ ID NO.5.

124. (Original) A diagnostic kit comprising at least one isolated and purified polypeptide comprising a WNV envelope (E) protein or an immunogenic fragment thereof having a native conformation or non-denatured structure whereby the E glycoprotein or the

immunogenic fragment thereof is reactive with antibodies against WNV and cross-reactive with antibodies against a flavivirus,

wherein, the diagnostic kit is an ELISA.

125. (Original) A method for detecting a recent or ongoing flavivirus infection using an immunoassay to detect an IgM antibody against a flavivirus in a biological specimen comprising the steps of:

(a) contacting the biological specimen with anti-IgG antibodies to form IgG immune complexes,

(b) removing said complexes to form a biological specimen comprising IgM antibodies but lacking IgG antibodies,

(c) contacting the biological specimen with a microsphere coupled to a substantially pure WNV E glycoprotein having a native conformation or non-denatured structure to form a microsphere mixture under conditions sufficient to form a binding complex between the E glycoprotein and a IgM antibody whereby the E glycoprotein is reactive to antibodies against WNV and cross-reactive to antibodies against a flavivirus,

(d) contacting the microsphere mixture with a detection reagent capable of detecting a IgM antibody,

(e) detecting the detection reagent,
whereby detection of the detection reagent indicates a recent or ongoing flavivirus infection,

wherein, the immunoassay is an ELISA.

126. (Original) A method for rapidly detecting an anti-WNV antibody in an animal by using an ELISA comprising the steps of:

(a) contacting a biological sample comprising at least one anti-WNV antibody with a reaction well surface, each reaction well surface coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against WNV but not detectably cross-reactive with antibodies against a flavivirus other than WNV,

- (b) incubating the biological sample under conditions sufficient to increase reaction kinetics to promote the binding of the at least one anti-WNV antibody to the NS5 protein,
 - (c) contacting the reaction well surface with a detection reagent capable of detecting an anti-WNV antibody,
 - (d) detecting the detection reagent,
- wherein detection of the detection reagent indicates the presence of the at least one anti-WNV in the biological sample.

127. (Original) A method for rapidly detecting a recent WNV infection in an animal by using an ELISA comprising the steps of:

- (a) contacting a biological sample comprising at least one anti-WNV antibody with a reaction well surface, each reaction well surface coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against WNV but not detectably cross-reactive with antibodies against a flavivirus other than WNV,
 - (b) incubating the biological sample under conditions sufficient to increase reaction kinetics to promote the binding of at least one anti-WNV antibody to the NS5 protein,
 - (c) contacting the reaction well surface with a detection reagent capable of detecting an anti-WNV antibody,
 - (d) detecting the detection reagent,
- wherein detection of the detection reagent indicates the presence of the at least one anti-WNV in the biological sample thereby indicating a recent WNV infection.

128. (Original) A method for rapidly determining whether a previously WNV-vaccinated animal recently sustained exposure to WNV by using an ELISA comprising the steps of:

- (a) contacting a biological sample of a previously WNV-vaccinated animal comprising at least one anti-WNV antibody with a reaction well surface, each reaction well surface coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against WNV but not detectably cross-reactive with antibodies against a flavivirus other than WNV,

- (b) incubating the biological sample under conditions sufficient to increase reaction kinetics to promote the binding of the at least one anti-WNV antibody to the NS5 protein,
 - (c) contacting the reaction well surface with a detection reagent capable of detecting an anti-WNV antibody,
 - (d) detecting the detection reagent,
- wherein detection of the detection reagent indicates a recently sustained exposure of the previously WNV-vaccinated animal to WNV.

129. (Original) A method for detecting a first antibody to a serospecific DENV from a biological specimen of a subject suspected of being infected by said serospecific DENV comprising the steps of:

- (a) contacting the biological specimen with a substantially pure serospecific DENV NS5 protein or an immunogenic fragment thereof having a native conformation and non-denatured structure under conditions to form a complex between the NS5 protein and the first antibody, if present, that recognizes and binds the NS5 protein,
 - (b) detecting the first antibody of said complex,
- wherein the NS5 protein is not detectably cross-reactive to an antibody against a flavivirus other than a DENV and is specific to the serospecific DENV.

130. (Original) The method according to claim 129, wherein said NS5 protein is coupled to a microsphere or adsorbed to nitrocellulose paper.

131. (Original) The method according to claim 129, wherein step (b) comprises the steps of:

- (b_i) contacting said complex between said NS5 protein and said first antibody with a second antibody reactive against said first antibody,
 - (b_{ii}) detecting the second antibody,
- wherein detecting the second antibody infers detecting the first antibody.

132. (Original) The method according to claim 131, wherein the second antibody includes a fluorescent marker or the second antibody is bound to colloidal gold or polystyrene microspheres.

133. (Original) The method according to claim 131, wherein the step of detecting the second antibody further comprises the step of immunofluorescence detection.

134. (Original) The method according to claim 131, wherein the second antibody is coupled to an enzyme which can be assayed.

135. (Original) The method according to claim 134, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

136. (Original) The method according to claim 129, wherein the biological specimen is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

137. (Original) A diagnostic kit comprising at least one isolated and purified polypeptide comprising a serospecific DENV NS5 protein or an immunogenic fragment thereof having a native conformation or non-denatured structure whereby the NS5 protein or the immunogenic fragment thereof is reactive with antibodies to a serospecific DENV and not detectably cross-reactive with antibodies against another flavivirus, wherein, the diagnostic kit an ELISA.

138. (Original) A method for rapidly detecting an anti-DENV antibody in an animal by using an ELISA comprising the steps of:

(a) contacting a biological sample comprising at least one anti-DENV antibody with a reaction well surface, each reaction well surface coupled to a substantially pure DENV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is

specifically reactive to antibodies against DENV but not detectably cross-reactive with antibodies against a flavivirus other than WNV,

- (b) incubating the biological sample under conditions sufficient to increase reaction kinetics to promote the binding of the at least one anti-DENV antibody to the NS5 protein,
 - (c) contacting the reaction well surface with a detection reagent capable of detecting an anti-DENV antibody,
 - (d) detecting the detection reagent,
- wherein detection of the detection reagent indicates the presence of the at least one anti-DENV in the biological sample.

139. (Original) The method according to claim 138, wherein the animal is selected from the group consisting of a monkey and a chimpanzee.

140. (Original) A method for rapidly detecting a recent DENV infection in an animal by using an ELISA comprising the steps of:

- (a) contacting a biological sample comprising at least one anti-DENV antibody with a reaction well surface, each reaction well surface coupled to a substantially pure DENV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against DENV but not detectably cross-reactive with antibodies against a flavivirus other than DENV,
 - (b) incubating the biological sample under conditions sufficient to increase reaction kinetics to promote the binding of at least one anti-DENV antibody to the DENV protein,
 - (c) contacting the reaction well surface with a detection reagent capable of detecting an anti-DENV antibody,
 - (d) detecting the detection reagent,
- wherein detection of the detection reagent indicates the presence of the at least one anti-DENV in the biological sample thereby indicating a recent DENV infection.

141. (Original) The method according to claim 140, wherein the animal is selected from the group consisting of a monkey and a chimpanzee.

142. (Original) A method for rapidly determining whether a previously DENV-vaccinated animal recently sustained exposure to DENV by using an ELISA comprising the steps of:

- (a) contacting a biological sample of a previously DENV-vaccinated animal comprising at least one anti-DENV antibody with a reaction well surface, each reaction well surface coupled to a substantially pure DENV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against DENV but not detectably cross-reactive with antibodies against a flavivirus other than DENV,
 - (b) incubating the biological sample under conditions sufficient to increase reaction kinetics to promote the binding of the at least one anti-DENV antibody to the NS5 protein,
 - (c) contacting the reaction well surface with a detection reagent capable of detecting an anti-DENV antibody,
 - (d) detecting the detection reagent,
- wherein detection of the detection reagent indicates a recently sustained exposure of the previously DENV-vaccinated animal to DENV.

143. (Original) The method according to claim 142, wherein the animal is selected from the group consisting of a monkey and a chimpanzee.

144. (Original) A method for rapidly detecting a recent or ongoing flavivirus infection in an animal susceptible of infection by said flavivirus, comprising the steps of:

- (a) contacting a biological sample comprising flavivirus antibodies of said animal with a microsphere suspension, each microsphere coupled to a substantially pure flavivirus NS5 protein having a native conformation or non-denatured structure whereby each flavivirus NS5 protein is reactive to said flavivirus antibodies but not detectably cross-reactive to antibodies against other flaviviruses,
- (b) incubating the microsphere suspension under conditions sufficient to promote the binding of said flavivirus antibodies to the flavivirus NS5 protein,
- (c) contacting the microsphere suspension with a detection reagent capable of detecting a flavivirus antibody,
- (d) detecting the detection reagent,

wherein detection of the detection reagent indicates the presence of said flavivirus antibody in said biological sample thereby detecting a recent or ongoing flavivirus infection.

145. (Original) The method of claim 144, wherein the flavivirus NS5 protein is a WNV NS5 protein and has the amino acid sequence of SEQ ID NO.8.

146. (Original) The method of claim 144, wherein the flavivirus NS5 protein is a DENV NS5 protein and has the amino acid sequence of SEQ ID NO.10.

147. (Original) The method of claim 144, wherein the flavivirus NS5 protein is a DENV NS5 protein and has the amino acid sequence of SEQ ID NO.12.

148. (Original) The method of claim 144, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

149. (Original) The method of claim 144, wherein the method for rapidly detecting a recent or ongoing flavivirus infection is in the form of an immunochromatographic test.

150. (Currently Amended) A method for carrying out an immunochromatographic test for rapidly detecting a flavivirus infection in an animal susceptible to said infection, comprising the steps of:

- (a) providing a membrane strip having a proximal end, a distal end, and a plurality of zones each comprising secondary antibodies coupled thereto,
- (b) providing a suspension of microspheres comprising flavivirus antigens coupled thereto,
- (c) providing a biological sample from said animal susceptible to said infection, comprising anti-flavivirus antibodies,
- (d) contacting the biological sample with the suspension of microspheres to form a reaction mixture under conditions sufficient to promote binding of said anti-flavivirus antibodies of said biological sample to said flavivirus antigens of the microspheres,

- (e) placing the reaction mixture at the proximal end of the membrane strip,
 - (f) incubating the membrane strip under sufficient conditions to promote the movement of the reaction mixture towards the distal end, said conditions also sufficient to promote the binding of the microparticles to said secondary antibodies coupled to the membrane strip vis-à-vis interactions between said secondary antibodies and said anti-flavivirus antibodies of said microparticles as the reaction mixture travels towards the distal end of the membrane strip,
 - (g) washing from the ~~the~~ membrane strip any unbound microparticles, and
 - (h) detecting bound microparticles,
- wherein bound microparticles indicates a flavivirus infection in said animal.

151. (Original) The method according to claim 150, wherein the animal is infected with DENV.

152. (Original) The method according to claim 150, wherein the animal is infected with WNV.

153. (Original) The method according to claim 150, wherein the animal is infected with JEV.

154. (Original) The method according to claim 150, wherein the animal is infected with SLEV.

155. (Original) The method according to claim 150, wherein the flavivirus antigen is a flavivirus NS5 antigen.

156. (Original) The method according to claim 150, wherein the flavivirus antigen is a WNV NS5 antigen with the amino acid sequence of SEQ ID NO.8.

157. (Original) The method according to claim 150, wherein the flavivirus antigen is a DENV NS5 antigen with the amino acid sequence of SEQ ID NO.10.

158. (Original) The method according to claim 150, wherein the flavivirus antigen is a DENV NS5 antigen with the amino acid sequence of SEQ ID NO.12.

159. (Original) The method according to claim 150, wherein the flavivirus antigen is a WNV E glycoprotein antigen with the amino acid sequence of SEQ ID NO.6.

160. (Original) The method according to claim 150, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

~~25.161.~~ (Currently Amended) The method according to claim 19, wherein said flavivirus is DENV.